UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: PP#1F3787. Abamectin (Avermectin B_1) for Use in/on Pears. Registrant's Response of 3/10/93 to Analytical Method Deficiencies Cited in J. Stokes Memo of 4/16/92. MRID# 426922-01. DP Barcode# D189398. CBTS# 11604.

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Merck and Co., Inc. is requesting the establishment of a permanent tolerance for abamectin (avermectin B_1) insecticide/miticide and its delta-8,9-isomer in/on pears at 0.035 ppm.

In the J. Stokes memo of 4/16/92, CBTS concluded that Method No. 8000 for the analysis of avermectin B_1 and 8,9-Z-avermectin B_1 residues in/on pears was not considered acceptable for enforcement purposes. The memo outlined six corrections/comments that should be addressed by the registrant before CBTS can make a decision concerning the adequacy of the method.

In the current submission, Merck has submitted a revised method (Method No. 8000, Rev. 4) and comments to the six corrections/comments made in the J. Stokes memo of 4/16/92.

Abamectin .035

Conclusions and Recommendations

The current submission adequately addresses the concerns CBTS had with the adequacy of the proposed enforcement method. The modified Merck Method No. 8000, Rev. 4 should be an adequate method for the enforcement of future abamectin tolerances on pears and apples. CBTS will send Method No. 8000, Rev. 4 to FDA to be published in PAM II.

Based on the J. Stokes memo of 11/26/92 concerning PP#1F3787, the registrant has not satisfied the requirement of submitting a revised Section F proposing a 0.05 ppm tolerance for the residues of abamectin on pears. **CBTS continues to recommend against the issuance of a permanent tolerance on pears until a new Section F is submitted.**

Detailed Considerations

The following Deficiencies/Comments were cited by CBTS in the 4/16/92 memo of J. Stokes concerning PP#1F3787. The responses from the registrant were received on 3/10/93.

Deficiency #1

Since the term "delta 8,9 isomer" can be a source of confusion in the discussion of avermectin residues, the petitioner is required to define these residues as 8,9-Z-avermectin B_{1a} and 8,9-Z-avermectin B_{1b} throughout the method.

Registrant's Response to Deficiency #1

Merck has submitted a revised method (Method No. 8000, Revision 4) to reflect the requested terminology (see Attachment I).

CBTS's Comments and Conclusions Concerning Deficiency #1

CBTS considers Deficiency #1 resolved.

Deficiency #2

You must provide the EPA repository with adequate analytical standards according to the Agency's requirements. We were unable to obtain purified analytical standards of avermectin B_{1a}, B_{1b}, or 8,9-Z-B_{1a} from the EPA Pesticides and Industrial Chemicals Repository (PICR) or from the petitioner. Instead, both PICR and Merck and Co. provided us with samples of dilute standard solutions of abamectin (0.956% B1a and 0.071% B1b in glycerol formal) and 8,9-Z-B1a (0.38% in glycerol formal).... instruct PICR on shipping requirements as well as the amount of diluted standard solution required in the method.

Registrant's Response to Deficiency #2

Abamectin drug substance (bulk technical or solid state) has two characteristics which make it unsuitable for routine use as a reference standard in laboratory analyses - it is a mixed, non-stoichiometric solvate and it is chemically unstable.

Abamectin drug substance contains up to 7.0% ethanol and 17.0% water. These solvents are not present in a fixed ratio (arising from defined solvates) and are therefore subject to facile variation (loss of ethanol and/or water, or uptake of water) depending on the environment (temperature and humidity) in which the drug substance is stored and handled. In addition, abamectin is not chemically stable and is subject to solid-state oxidative decomposition.

Both of the unfavorable characteristics have been overcome through the development of an abamectin glycerol formal solution for use as a routine laboratory reference standard. Abamectin, and associated ethanol and water, are completely soluble in glycerol formal at the concentration employed. Glycerol formal is non-volatile and non-hygroscopic, and therefore, solvation variations after dissolution of abamectin are eliminated. In addition, glycerol formal has desirable stabilization properties and inhibits the oxidation degradation of abamectin.

When the abamectin glycerol formal solution was prepared, the B1a and B1b isomer concentrations were accurately determined versus a specially prepared solid reference lot which is no longer available (because of the unfavorable characteristics previously mentioned). The solution was subdivided into individual amber glass containers, each with an amount convenient for multiple analyses, and stored frozen to insure stability. The solution is dilute, permitting the accurate weighing of a convenient amount which does not require excessive dilution to prepare working standards with concentrations appropriate for use in trace residue analyses. Refrigerated, or preferably frozen, shipment and storage is desirable to maintain the standard's integrity.

The abamectin glycerol formal solution standard is suitable for its intended use, and has been successfully employed by several Merck laboratories and numerous contract laboratories which conduct residue analyses both in the US and internationally. The glycerol formal standard solution is of defined purity and sufficiently concentrated for all residue determinations, including the method described for pears. Finally, there is no solid abamectin standard that is available or suitable for use.

As might be expected from the similarities in the structure, the avermectin B1a delta 8,9-Z isomer has similar characteristics. Consequently, a solution of avermectin B1a delta 8,9-Z isomer standard in glycerol formal has been prepared and is used. However, we have determined that the avermectin B1a delta 8,9-Z isomer yields the same derivative as is obtained from the parent

avermectin B1a so that it is not necessary to use the delta 8,9-Z isomer standard, except during the initial validation of the method

CBTS's Comments and Conclusions Concerning Deficiency #2

Based on the inherent properties (unstable, hygroscopic) of the abamectin standards, the concentration levels of the supplied standards relative to the proposed tolerance level in pears (0.035-0.05 ppm), and the process by which abamectin is manufactured (fermentation process using a strain of Streptomyces avermitilis), CBTS considers the supplied standard solutions in glycerol to be adequate for enforcement purposes. **Deficiency #2 is resolved.**

Deficiency #3

The use of the B1a calibration curve to quantitate both B1a and B1b is not analytically correct, and will not be acceptable unless the two analytes are demonstrated to produce equivalent HPLC responses in the method.

Registrant's Response to Deficiency #3

Avermectin B1b is at most 20% and usually less than 10% of the avermectin content in the formulation and in the incurred residue. Avermectin B1a is at least 80% and usually more than 90% of the avermectin residue. Consequently, the B1b residues are usually not quantifiable and generally not even detectable at the PHI, no matter which calibration curve is used.

B1a and B1b differ by one methylene group connected at the C-25 position. Although B1a and B1b are resolved chromatographically in a reverse phase HPLC system, the quantitation is based on the fluorescent derivative response. The fluorescent part of the molecule is in the extended conjugation associated with the aromatized ring, which is the same for avermectin B1b and B1a. We have demonstrated the equivalence of the response for B1b to B1a and have previously provided documentation (see Attachment II). The pear method uses the same fluorescent derivative as discussed in Attachment II and the matrix does not present any interferences to affect the fluorescence sensitivity, as illustrated in the validation of the method for B1a and B1b.

CBTS's Comments and Conclusions Concerning Deficiency #3

Although not analytically correct, Merck has provided sufficient data to show that the quantitation of avermectin B1b residues using the B1a curve will accurately measure the contribution of B1b in the total avermectin residue up to approximately 100 ng/g (ppb) total. Since the proposed tolerance level in pears is 35-50 ppb (i.e. <100 ppb), and the pear matrix has been shown not to present any interferences that would affect the fluorescence sensitivity, CBTS considers Merck Method No. 8000, Rev. 4 to be an adequate method for the enforcement of avermectin residues on pears. **CBTS considers Deficiency #3 resolved.**

Note: If the need arises to raise the tolerance level on pears above 100 ppb, or if Method No. 8000, Rev. 4 is utilized for other commodities (especially other commodities whose tolerance levels exceed 100 ppb or if interferences are seen or expected), Merck will need to provide additional validation.

Deficiency #4

Merck's Method No. 8000 and "Suggestions for the Analyst Performing Merck Residue Method No. 8000" (dated 11/18/87) must be combined into a single document.

Registrant's Response to Deficiency #4

Method 8000 has been revised (Method 8000, Rev. 4) to incorporate the suggestions for the analyst into the text of the method. The method has also been revised for other reasons. The reasons are described in the revision histories in the back of the method (Attachment I). Most of the revisions are minor and for clarification. However, more significant revisions have been made in the method description to reflect the extension or application, including validation, of the method to apples. Also the method has been validated to quantitate to a lower level, going from the 5 ng/g originally specified to 2 ng/g. The limit of detection has been lowered to 1 ng/g. This applies to both pear and apple matrices. None of the revisions affected the experimental procedure employed, except in the quantitation.

CBTS's Comments and Conclusions Concerning Deficiency #4

Based on the nature of the changes in the method for pears, as well as the supplied recovery values and chromatograms for apples (another pome fruit), CBTS considers Method No. 8000, Rev. 4 to be an adequate method for the enforcement of avermectin residues on pears and apples. **CBTS considers Deficiency #4 resolved.**

Comment #5

The limit of detection for this method was not determined by the EPA, but it appears to be less than 1.0 ppb. Percent recoveries for samples of pears fortified with 32.6 ppb of avermectin B1a were 94 and 102....

Registrant's Response to Comment #5

The limit of detection has been redefined to be below 1 ng/g, based on additional experience and validation of the method, including quantitation down to 2 ng/g.

CBTS's Comments and Conclusions Concerning Comment #5

No comment necessary. This was never a deficiency.

Deficiency #6

Method required a minimum of two full days to prepare and complete analyses of fortified samples. Unknown samples may require a repeat run for quantitation if these are not in the narrow calibration curve. The procedure marginally meets the guidelines for analysis within 24 hours provided a repeat analysis is not required.

Registrant's Response to Deficiency #6

Unknown samples which are above the calibration curve need to be rediluted and reinjected on the HPLC; they do not need to be reassayed. If unknown samples are below the calibration curve, they have no detectable residue.

CBTS's Comments and Conclusions Concerning Deficiency #6

For enforcement purposes, the scope of interest concerns a narrow concentration range near the tolerance level. CBTS considers Method 8000, Rev. 4 adequate for enforcement purposes. **CBTS considers Deficiency #6 resolved.**

Note: For the purposes of generating monitoring data, Method 8000, Rev. 4 may be useful provided additional standards levels are utilized, especially at the lower end. If the response of the standards turns out not to be linear, the samples can be quantitated based on a single standard that is close in response to sample.

Attachment I - Method No. 8000, Rev. 4

Attachment II - letter and data demonstrating the equivalent response in B1a and B1b

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